

# **NREL-Amoco CRADA Phase 3**

## **Bench Scale Report 1.4**

### **Growth and Xylose Utilization by L1400(LNHST2)**

#### **Propagated on Glucose and Xylose**

**Project Title:** NREL-Amoco CRADA with Corn Fiber

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#### **Objective**

Measure the growth rate of the yeast strain LNHST2 on glucose in order to develop the PDU seed train protocol and determine if xylose utilization is affected by serial propagation on glucose (Note: In the rest of the report, L1400(LNHST2) is referred to as ST2 and L1400(pLNH33) as LNH33.)

#### **Background**

The PDU seed train consists of five stages. The first two stages are carried out in the laboratory starting at 100 mL. From there, the seed volume increases in 10-fold increments until the final stage, which consists of 1,000 L of seed. In order to determine the time line and staffing requirements for propagating seed for PDU runs, the growth rate of ST2 has to be ascertained.

In addition, the question as to whether or not the yeast can be propagated on glucose without affecting its ability to utilize xylose (during the subsequent SSCF process) has to be investigated. This is of concern, since previous xylose fermenting L1400 strains (plasmid-based) required selection pressure by xylose to retain their ability to ferment that sugar. The xylose catabolism genes in ST2 have been incorporated into the genome and, therefore, xylose utilization should not be as strongly affected by inoculation on glucose as it was with previous xylose fermenting strains, where the trait was plasmid-borne. This is critical to both the PDU and the commercial operation, since supplying xylose to the seed tanks would be a costly and difficult to handle task, and because xylose degrades when subjected to heat sterilization.

#### **Materials and Methods**

##### *Inoculum Preparation*

The inoculum was prepared by streaking a colony from a YEPX plate (1% w/v yeast extract, 2% w/v peptone, 2% w/v xylose and 2% w/v agar, pH 5.0) containing colonies from the original stock of ST2 received from Nancy Ho onto a fresh YEPX plate. The plate was incubated at 30°C for 48

hours, at which time a loopfull was inoculated into 100 mL of YEPD (1% w/v yeast extract, 1% w/v peptone, 2% w/v glucose, pH 5.0) generating the first of five flasks to be grown on glucose.

In order to determine the effect that propagation on glucose may have on xylose utilization, ST2 was serially transferred every 12 hours for a total of five times on glucose in YEPD. In addition to being inoculated in YEPD, cultures from the first, third and fifth flasks were also inoculated in flasks containing either YEPX (1% w/v yeast extract, 1% w/v peptone, 2% w/v xylose, pH 5.0) or CSL-X (1% w/v corn steep liquor (CSL), and 2% w/v xylose, pH 5.0). All 11 flasks that were used to carry out this experiment were inoculated with a 10% v/v inoculum in 90 mL of media in 500 mL baffled flasks and incubated at 30°C and 150 rpm agitation.

### *Sample Protocol*

Glucose and ethanol values were obtained from the YSI for the initial and final samples at a minimum on every flask. The optical density (OD), measured at 600 nm, was monitored in each flask to determine the growth profile under the various conditions. However, as the YEPD flasks were inoculated every 12 hours, the growth profiles in YEPD were only performed on those flasks inoculated in the morning for practical reasons. The pH of each sample was monitored with a calibrated external pH probe.

Every sample was submitted to the CAT Task and analyzed for glucose, xylose, xylitol, succinic acid, lactic acid, acetic acid, and glycerol by HPLC and ethanol by GC.

### **Results and Discussion**

The primary focus of this experiment was to determine the growth rate of ST2 on glucose and determine if growth on glucose has an ensuing effect on xylose utilization. After the cells were cultured on glucose one time, the growth rate in YEPX was 0.259 h<sup>-1</sup> and slightly slower on CSL-X at 0.236 h<sup>-1</sup>. By the fifth subculture on glucose, however, the growth rates on the xylose media had declined to 0.178 h<sup>-1</sup> on YEPX and 0.163 h<sup>-1</sup> on CSL-X (Table 1). This is a significant (31%) reduction in growth rate.

**Table 1:** Growth rate values, doubling times, and glucose and xylose utilization for ST2.

Flask	Media	Time of Inoculation	Growth Rate (h <sup>-1</sup> )	Doubling Time (h)	Glucose Utilization (g/Lh)	Xylose Utilization (g/Lh)
1	YEPD	pm	-	-	-	-
2	YEPD	am	0.394	1.76	5.12	-
3	YEPX	am	0.259	2.68	-	1.14
4	CSL-X	am	0.236	2.94	-	1.12
5	YEPD	pm	-	-	-	-
6	YEPD	am	0.339	2.04	4.91	-
7	YEPX	am	0.191	3.63	-	0.99
8	CSL-X	am	0.179	3.87	-	0.90
9	YEPD	pm	-	-	-	-
10	YEPX	am	0.178	3.89	-	1.39
11	CSL-X	am	0.163	4.25	-	1.31